C1q: first component of classical complement pathway facilitates *Streptococcus pneumoniae* adherence and invasion of host cells

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ABSTRACT:

Streptococcus pneumoniae (the pneumococcus) is a major human pathogen affecting both adults and children. Pneumococci have evolved numerous successful strategies to colonize the host. Here we report a novel mechanism of pneumococcal pathogenesis, whereby pneumococci utilize a host protein, primarily involved in host defence mechanism, for colonization and subsequent dissemination. Our data demonstrate a hitherto unknown function of C1q, the subunit of the first component of classical complement pathway, in the interaction between pneumococci and host cells. Using cell-culture infection assays and confocal microscopy we observed that pneumococcal surface bound C1q significantly enhanced pneumococcal adherence to and invasion of host epithelial and endothelial cells. Using flow cytometry, we demonstrated a direct, antibody-independent binding of purified C1q to various clinical isolates of pneumococci. This interaction was seemingly capsule serotype independent and mediated by the bacterial surface exposed proteins as pre-treatment of pneumococci with pronase E but not sodium periodate significantly reduced C1q binding. Moreover, similar binding was observed using C1 complex as the source of C1q. Furthermore, our data show that C1q bound to the surface of pneumococci through the globular head region and with the host cell surface receptor(s)/glycosaminoglycans via its N-terminal collagen-like stalk, as the presence of C1q N-terminal fragment and low molecular weight heparin but not the C-terminal globular heads blocked C1q mediated pneumococcal adherence to host cells. Taken together, we demonstrate for the first time a unique function of complement protein C1q, apparently unrelated to its host defense function, as a molecular bridge between pneumococci and the host, which promotes bacterial cellular adherence and invasion.

Pneumolysin and LytA avoid phagocytosis by alveolar macrophages and neutrophils and enhance invasive disease

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ABSTRACT:

Leukocytes play an important role in inflammatory and immune responses. Clear-ance of the bacterium depends on the efficacy of different receptors on the surface of phagocytic cells to recognize, internalize and kill the pathogen [1]. Phagocyto-sis of *Streptococcus pneumoniae* was greatly enhanced in the absence of pneumo-lysin and LytA demonstrating a synergistic effect by both proteins in host immune response evasion. A novel interaction with the P-selectin glycoprotein ligand-1 receptor (PSGL-1) of neutrophils was observed, with a significant involvement of Ply and LytA in this process. Moreover, bacterial levels were significantly in-creased in PSGL-1–/– knockout mice confirming the importance of this receptor in the recognition and clearance of *S. pneumoniae*.

Keywords: Phagocytosis, neutrophils, PSGL-1, mice.

1. Introduction

Although the capsular polysaccharide plays a major role in virulence by avoiding the host immune system, there are several pneumococcal proteins that are involved in different steps of the pathogenesis process [2]. One of these proteins is the cell wall hydrolase (au-tolysin) LytA. This protein is suggested to be involved in virulence by releasing pneumolysin (Ply) and cell wall fragments that are markedly pro-inflammatory. Ply is a toxin partially responsible for immune response evasion by interacting with the C1q complement component [3]. Inflammatory processes are dependent on the recruitment and activation of immune cells which in turn require the formation of intracellular contacts involving cell adhesion molecules [4,5]. Expression of P-selectin and E-selectin by the endothelium is involved in the rolling process and also supports protection against invading pathogens such as *S. pneumoniae* [6]. The PSGL-1 receptor has been described as a functional leukocyte receptor for the invasion of different intracellular microorganisms such as enterovirus or *Anaplasma phagocy tophilum* [7].

2. LytA and pneumolysin divert phagocytosis by alveolar macrophages

The adhesion to alveolar macrophages was slightly increased in the absence of Ply or LytA and higher for the double mutant suggesting that these two proteins avoid the recognition of *S. pneumoniae* by alveolar macrophages. Phagocytosis of the single mutants was enhanced compared to the phagocytosis of the wild-type strain whereas the

double mutant strain was strongly phagocytosed in comparison to the single mutants confirming that these two proteins avoid very efficiently the phagocytosis of *S. pneumoniae*. Confocal microscopy assays confirmed co-localization of the bacteria with early and late endosomal markers demonstrating that loss of Ply and LytA increased the efficiency of macrophages to process the bacteria through their phagolysosomal pathway.

3. PSGL-1 receptor plays an im-portant role in the phagocytosis of *S. pneumoniae*

The biological role of the PSGL-1 receptor in the uptake of *S. pneumoniae* by neutrophils and the involvement of Ply and LytA in the evasion of this process was investigated. Phagocytosis of *S. pneumoniae* was significantly increased when the PSGL-1 receptor was present with higher levels for the different mutants in comparison to the wild-type strain. When the PSGL-1 receptor was blocked, a significant reduction in the phagocytosis of *S. pneumoniae* was found, with a dramatic effect in the ply and *lytA* mutants. Moreover, confocal microscopy experiments demonstrated that the different strains colocalized with PSGL-1 receptor and this interaction requires phosphorylation of the Syk kinase.

4. PSGL-1 reduces the severity of pneumococcal infection

In a pneumonia model of infection, bacterial levels of the wild-type strain were slightly higher in BALF, but not in the lung of PSGL-1–/– mice. This effect was more dramatic in the blood suggesting that PSGL-1 contributes to control bacteria replication in the systemic circulation reducing the severity of bacterial dissemination from the lung to the bloodstream. In a sepsis model of infection, mice lacking PSGL-1 had greater levels of bacteria in blood and the lethal infection developed faster in PSGL-1–/– mice confirming that this receptor plays a critical role in host defense against IPD.

5. Conclusion

These results together demonstrate that Ply and LytA impair the recognition and phagocytosis of *S. pneumoniae* by professional phagocytes and demonstrate a novel role for PSGL-1 in host defense against pneumococcus.

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Within host selection and evolution of *Streptococcus pneumoniae* during acute invasive infection

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ABSTRACT:

Pathogenesis of pneumococcal invasive infection is complex and the balance of host and microbial factors play a pivotal role. We use a model of intravenous infection with strain TIGR4, where the challenge dose of one million bacteria is reduced by initial macrophage clearance to a minimum and invasive infection initiates from one or very few cells. We used whole genome sequencing to investigate the dynamics of the pneumococcal population during invasive infection. Sequencing of pneumococci recovered from blood cultures confirmed the monoclonal origin of bacteraemia by identification of one single SNP in 100% of bacteria isolated from the host. The identified SNPs are not linked to virulence and the escape of bacteria from immune mechanisms is a stochastic event. In addition pneumococcal populations in the majority of mice showed sub-populations characterized by SNPs in different subunits of F0/F1 ATPase operon. Pneumococci with mutation in the F0/F1 ATPase operon show improved growth at acidic pH, impaired growth at basic conditions, changes in surface characteristics and increased counts in spleen of infected mice (see abstract Gerlini et al., this meeting). This work provides genetic evidence for a monoclonal origin of infection and underlines the strong selection on the pneumococcal population during invasive infection.